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Aggregate Stabilization of Volcanic Ash and Soil During Microbial Degradation of Straw†

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Bacterial and fungal colonists of straw in soil promoted aggregate stabilization of volcanic ash and soil.

The Palouse soils of the Pacific Northwest of the United States are both volcanic and alluvial in origin and are unstable and subject to severe water erosion. On 18 May 1980, Mount St. Helens erupted and covered much of the region with volcanic ash, which is analogous to new parent material and, hence, extremely unstable, thus adding to the instability of the soils of the area. We took the opportunity offered by this event to study some of the factors that might contribute to stabilizing soil.

Although reviews show that microorganisms can stabilize soil aggregates (2, 4, 7, 11), there has been relatively little interest in this field in the past decade, except for extensive studies on the interactions between clay particles and microorganisms (12). Furthermore, little attempt was made in the past to relate this effect to the size of the biomass or, with exceptions (3, 5, 10), to the available substrates in soil. Microbial products, particularly polysaccharides (8, 9, 11), are likely agents of aggregation. Generally, both aggregation, which is the coalescence of particles to form aggregates, and water stability, which is a measure of the resistance of the aggregates to slaking in water, now appear to be related to the size of the biomass in soil (9). However, in one unstable soil, a common soil fungus, *Mucor hiemalis*, reduced stability (9).

The addition of microbially degraded straw residues containing aggregating agents, e.g., composts, is likely to be beneficial to unstable soils. The present study showed, in laboratory experiments, the effect of the addition of such straw residues on the increase in aggregate stability of volcanic ash and soil and the lack of effect of this procedure on water retention by the aggregates. Scanning electron microscopy demonstrated the position of the microorganisms and their products between the particles of the aggregates.

Particles. Unleached volcanic ash and soils (mixed mesic, pachic, and Ultic Haploxerolls) with different cropping histories were collected from Pullman, Wash., and sieved to pass a 3-mm screen. The respective percentages of sand, silt, and clay were 22.2, 64.2, and 13.6 (ash); 13.2, 72.8, and 14.0 (soil A); and 13.2, 64.6, and 20.2 (soil B); therefore, they all were silt loams. The pHs (for particles diluted 2.5:1 in water) were 6.1 (ash), 5.8 (soil A), and 5.1 (soil B). Soil A had 1.46% (wt/wt) organic C and had been in continuous wheat for 2 years. Soil B had 1.13% (wt/wt) organic C and had a barley-pea rotation in the previous 2 years.

Substrates and inocula. *Azotobacter* sp., *Enterobacter cloacae, Mucor plumbeus, Penicillium purpurascens*, and *Trichoderma harzianum* were isolated from decomposing straw by using a soil inoculum. The bacterium (*E. cloacae*) was grown in Oxoid nutrient broth, and the fungi (*M. plumbeus, P. purpurascens*, and *T. harzianum*) were grown in Oxoid malt broth. All were grown axenically in conical flasks (capacity, 325 ml) containing 100 ml of medium on a rotary shaker at 200 rpm and 20°C for 4 days. The cells or mycelia were centrifuged and washed in sterile distilled water three times before they were added to the ash.

Both bacteria and the three fungi, either singly or in combination, were inoculated non-axenically into a medium containing wheat (*Triticum aestivum* L.) straw (1 g), milled to pass a 0.4-mm screen, in 100 ml of mineral salts (1). The cultures were incubated at 200 rpm on a rotary shaker for 25 days. Periodically, samples of the incubated suspensions were removed to add them to the ash or soil.

Physical measurements. To investigate the direct effect of microbial biomass on the ash, cells

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or mycelia in a suspension (10 ml) were added to the ash (10 g) in petri dishes (85-mm diameter). The mixtures were dried immediately at 60°C for 3 h. For each ash sample, five subsamples (0.75 g each) were taken and placed in a boiling tube (capacity, 100 ml). Deionized water (60 ml) was added, and the aggregate stability was determined by shaking and measuring the turbidity of the suspension after 1 min (9).

To investigate the effect of straw on the water stability of ash and soil, samples (10 g) of dry soil or ash were treated in petri dishes (85-mm diameter) with 10 ml of the degraded or undegraded straw suspensions in mineral salts, fresh mineral salts medium, or deionized water. The mixtures were either incubated at 25°C for 7 days before being dried or dried immediately at 60°C for 3 h. At the start of the 7-day incubation period, the soil was water saturated, but at the end it was air dry; the soil thus experienced a range of water regimes. From each dried soil, at least five subsamples (0.1 g each) were taken, each of these was added to deionized water (20 ml) in a tube that was then inverted by hand 10 times, and the turbidity of the suspension was recorded after 1 min at 660 μm with a Beckman model B spectrophotometer.

Calibration curves for the ash and soil were prepared based on the negative correlation of the percent transmission with the weight of silt and clay in suspension, i.e., a positive correlation with stability. Thus, aggregate stability could be estimated by use of the calibration curve. Moisture retention of the aggregates was determined by placing them overnight on sintered glass at a suction of 113 cm of water, using a hanging column of water. Four replicates were used for each determination.

Microscopy. Samples of the dried aggregates were broken apart, coated with gold twice, and examined by scanning electron microscopy.

The addition of *E. cloacae* cells to the ash made it completely water stable (Table 1). Although this is not a commonly studied soil bacterium, we have frequently isolated it from decomposing straw in soil. Of the fungi, only *T. harzianum* and, to a much lesser extent, *P. purpurascens* were effective. The statistical analysis was carried out on the turbidity measurements before the results were expressed as percentages of soil in suspension.

The addition of the microbially degraded straw to the ash and to both soils increased water stability, but there was no consistent advantage of the inoculants over the straw which had been degraded by the microflora naturally associated with it (Table 2). The stabilizing effect of the degraded straw on both soils was similar, even though one soil had a greater soil organic matter content.

TABLE 1. Effect on aggregation of microbial biomass added to ash

Microorganism	Biomass (mg per g of ash)	% Ash in suspension[a]
E. cloacae	6.2	0
	12.4	0
M. plumbeus	7.9	84
	15.8	68
P. purpurascens	8.3	71
	16.5	55
T. harzianum	4.5	73
	8.9	1
None	0	84

[a] Least significant difference ($P = 0.05$), 20%.

The results in Table 3 for the degraded straw are the means of 180 and 270 determinations on the ash and soil, respectively, for treatment with individual or pairs of microbial species after 6, 12, 19, and 25 days of straw degradation. Degradation for 6 days produced the full effect, and the extent of this effect did not differ significantly between microorganisms. Furthermore, when the ash or soil was incubated with the degraded straw suspensions for 7 days before determination of aggregate stability, there was no additional effect on aggregate stability over that which had been measured initially.

The water retention of soil A was significantly less than that of the ash, whether or not straw was added. The undegraded straw had a negative effect, whereas the degraded straw increased moisture retention, but the effects were small and were significant only with the ash (Table 3). The ash retained more water than the soil because it was filled with pores (G. S. Campbell, personal communication); i.e., it had a low particle density. When the native organic matter was removed from the soil by mild oxidation with hydrogen peroxide, the water retention was decreased to 0.278 g per g of soil. Thus, whereas plant residues are the initial substrates in humification, they affect water retention by aggregates less than do the humic materials that result from microbial degradation.

Fungal hyphae (Fig. 1a) and gums presumed to be of microbial origin (Fig. 1c) in soil treated with straw inoculated with *T. harzianum* were evident when the aggregates were broken apart. Bacteria on straw which had been inoculated with *E. cloacae* and added to ash were also evident (Fig. 1b).

It appears that microbial cells and polymers, but not straw, are among the agents of aggregate stabilization. The ash, with no inherent stability,

TABLE 2. Water stability of ash and soil aggregates formed by addition of straw inoculated with microorganisms

Inoculant[a]	% Solid in suspension		
	Ash	Soil A	Soil B
Azotobacter sp.	29.5	8.3	7.9
E. cloacae	21.5	8.0	7.5
M. plumbeus	22.5	7.8	9.5
P. purpurascens	16.5	7.0	9.0
T. harzianum	21.0	6.0	5.0
Azotobacter sp. + *T. harzianum*	21.5	7.9	7.9
E. cloacae + *T. harzianum*	25.0	9.5	8.3
M. plumbeus + *P. purpurascens*	21.0	8.3	7.8
None[b]	20.0	7.0	6.7
Undegraded straw in mineral salts	36.3	11.0	10.5
Soil only[c]	42.5	15.5	13.5
Least significant difference ($P = 0.05$)	8.5	1.8	1.8

[a] Straw was degraded for 19 days before ash treatments and for 25 days before soil treatment.
[b] Mineral salts medium only was added to ash or soil; straw was not sterilized. Decomposition was by natural microflora only.
[c] Deionized water only was added to soil.

provided a useful model to quantify the role of biomass and substrates in stabilizing soil. Although it is difficult to compare different studies directly because very small differences in methodology can produce widely varying values of water stability, it is interesting that similar added biomasses were effective in an earlier study (9). In that study, it was found that another *Mucor* species was ineffective; indeed, it inhibited the stabilizing process. *Mucor* species are generally non-cellulolytic, whereas *T. harzianum* and *P. purpurascens*, which were effective in the present study, are cellulolytic. However their cellulolytic activity did not appear to confer an advantage in the utilization of straw as a substrate to increase its stabilizing potential. Presumably they could not compete successfully with the natural microflora on straw, or, alternatively, the natural microflora was just as effective in producing aggregate stability.

Straw only becomes available slowly as a substrate to microorganisms, and therefore it does not support large increases in associated biomass; more likely, it provides substrates for the maintenance requirements of a very small increase in biomass. This probably explains why the aggregating effect was as great after 25 days as after 6 days of incubating straw with microorganisms in culture.

From the results obtained, it is not possible to determine whether microbial cells, excreted microbial products, or products released during death were the agents of aggregation. Between particles of aggregates determined to be water stable, Fig. 1a clearly shows a fungal hypha, which is intact apart from some holes that might have resulted from predator action, whereas Fig. 1c shows a structure between the soil particles which could be an extracellular polymer or, as it has similar dimensions to the hypha, might have originated from lysis, either naturally or as a result of the drying procedure.

The stabilizing effect of microorganisms is likely to be sustained as straw decomposition in the field continues during the growing season (6), even though some components of the soil biomass will eventually decompose the aggregating agents (11).

TABLE 3. Aggregate stability and water retention of ash and soil aggregates

Treatment	% Solid in suspension[a]		Water retention (g per g of solid)[a]	
	Soil A	Ash	Soil A	Ash
Degraded straw	8.5*a*	25.0*c*	0.379*w*	0.461*x*
Undegraded straw in mineral salts	11.0*b*	38.2*d*	0.370*w*	0.437*y*
None (control)[b]	13.6*b*	40.5*d*	0.378*w*	0.449*z*

[a] Values not followed by the same letter are significantly different ($P = 0.05$) by Duncan's multiple range test for a variable sample.
[b] Sterile mineral salts medium only was added to ash or soil.

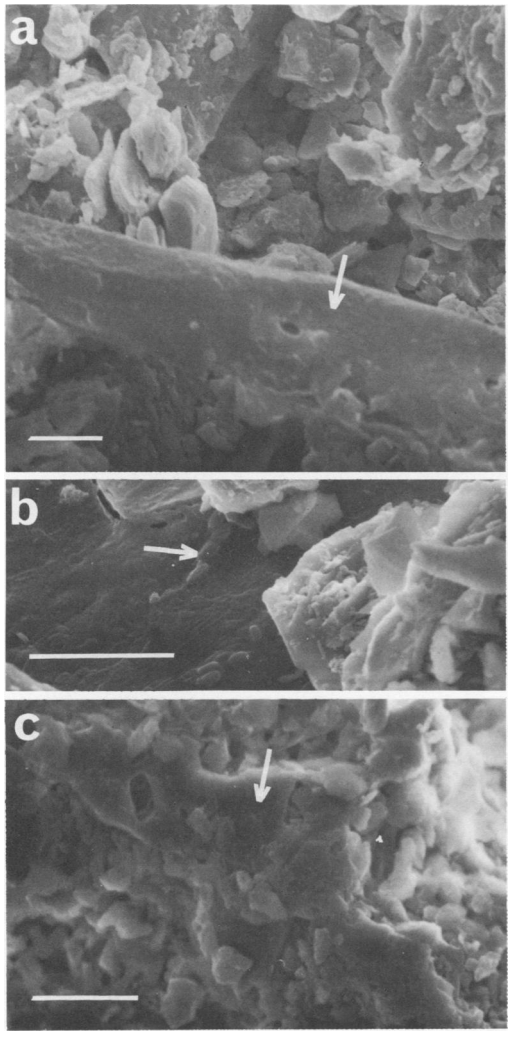

FIG. 1. (a) Fungal hypha with adhering soil. (b) Bacteria on the surface of straw with adhering ash. (c) Microbial products with adhering soil. Arrows indicate microorganisms and products. Bars, 10 μm.

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